Translocation Program Attachment 2

Excerpts from Karl 2002b

DESERT TORTOISE ABUNDANCE IN THE FORT IRWIN NATIONAL TRAINING CENTER EXPANSION AREA: SECOND-YEAR STUDIES

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EXECUTIVE SUMMARY

In an ongoing effort to examine impacts to desert tortoises that could occur from the expansion of Fort Irwin's National Training Center (NTC), the U.S. Department of the Army (Army) directed studies in 2001 and 2002 to examine tortoise density. Studies were concentrated in the western expansion area (Superior Valley area) where the most intensive military training is anticipated, with more limited sampling in the southern portion of the expansion area, in the area (9-0 Area). In 2001, six 1.0 km² mark-recapture plots and one 1.44 km² mark –recapture plot were completed in Spring, 2001 employing a single mark and recapture Peterson model. In addition, 568 belt transects were completed. Because estimated tortoise densities on all of the plots were low to very low, 0-14 tortoises/ km² (0-36 tortoises/mi²), statistical confidence intervals around the point density estimates were large. Since the point density estimates on the plots were the basis for determining tortoise densities on the subsequent transects and the resultant estimate of total tortoises in the western expansion area, it was necessary to demonstrate that the point estimates for the 2001 plot studies were reasonably accurate, despite the low sample size. In 2002, two plots were surveyed using multiple plot coverages (at least three), thus permitting the use of another statistical model, the Schnabel multiple-recapture model, to determine tortoise density, in addition to a Peterson estimator. One of the plots, Plot 8, had been surveyed in 2001. Another 116 transects were also conducted over portions of the 9-0 Area and the Superior Valley Area where 2001 results suggested that further transects were warranted or where the data were unusable in. The results of the 2002 studies are reported here.

The repetition of Plot 8 confirmed the previous year's result for this plot. In 2001, the Peterson density estimate was 12.0 tortoises per km² (4.03-32.06). Both this point estimate and confidence interval were nearly identical to the 2002 Peterson estimate, 10.8 tortoises per km² (4.29-23.60). The multiple recapture model (Schnabel) resulted in nearly the same point estimates as the single recapture model (Peterson), 11.6 tortoises per km² (6.34-19.27). Multiple recaptures succeeded, however, in narrowing the confidence interval for the density estimate. Both the repeatability evident in the results from 2001 to 2002 and the repeatability among results obtained using different models strongly suggest that the density estimates on the mark-recapture plots completed in 2001 are valid. Further, the point estimates on which the transect indices, and ultimately the abundance in the expansion area were based, can be assumed to be approximately correct.

Of the 258 km² in the western proposed expansion area, we now have sampled 130 km² by either transects or mark-recapture plots (Years 2001 and 2002, combined). While this is 50.4 % of the kilometers in the entire western area, the concentration of transects resulted in a sampling of 83 % of the main-use area. For the entire western expansion area the total number of adults, in both sampled and unsampled kilometers, is estimated at 563-595 tortoises (approximate confidence interval: 531-1158). Nearly 50 % of the western expansion area was estimated to have 1 or fewer tortoises/km² (3 tortoises/mi²) and less than 2 % was estimated to have more than 10 tortoises/km² (>26 tortoises/mi²). There were no kilometers where estimated densities exceeded 14 tortoises/km² (36 tortoises/mi²).

We have sampled 71 % of 9-0 Area north and west of Alvord Mountain. Cumulative estimated adult tortoise density, from 2001 and 2002 studies, for the sampled kilometers is 269-278 adult tortoises. For the entire 45 km² area, the total number of adults, in both sampled and unsampled kilometers, is estimated at 386-395 tortoises (approximate confidence interval: 293-595).

Current densities in the Superior Valley Area are low to very low and it is likely that densities were probably never high there, based on habitat and elevational influences. By contrast, the 9-0 Area has moderate densities that are more consistent over the area and better habitat than the western expansion area, so it may have supported higher densities than it currently does. Current densities are likely to be depressed due to high mortality and depressed reproduction associated with the recent, 14-year drought.

The current study incorporated numerous improvements in both the transect and plot methodologies, including increasing sampling intensity from 1.47 % to 14.7 % of a square kilometer, consideration of tortoise size, using appropriate sign types for the density index, truncating data by sign type and observer, maintaining identical sampling intensity per kilometer for both calibration transects and expansion area transects, and choosing appropriate plots for calibration transects. All of these factors resulted in high regression correlations for the index (i.e., r² values), thus providing high confidence in the ability of these transects to predict tortoise density. This, combined with the year-to-year consistency in the transect results strongly validates the reliability of the revised techniques.

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DESERT TORTOISE ABUNDANCE IN THE FORT IRWIN NATIONAL TRAINING CENTER EXPANSION AREA: SECOND-YEAR STUDIES

1.0 INTRODUCTION

The United States Department of the Army (Army) has proposed to expand the National Training Center (NTC) at the Fort Irwin Military Reservation (Fort Irwin) north of Barstow, California, for over a decade. Congress approved a joint plan between the Army and the Department of the Interior (Interior) for the expansion (H.R. 5666) and it was signed by President Clinton on 21 December 2000. The Army then submitted a plan that would identify those lands that the Army considers essential for more effective maneuver training. Historically, these have included areas south, southwest and east of the NTC, although areas to the northeast were also considered (http://www.fortirwinexpansion.com). The currently proposed expansion area includes 25,779 ha (63,673 acres) to the west of the current NTC boundary, in an area commonly called the Superior Valley Area, and 18,801 ha (46,438 acres) to the east, in the Eastgate Area (Figure 1). In addition, approximately 8900 ha (22,000 acres) in the southern portion of the expansion area, the "9-0 Area" will be re-incorporated into use. This latter area has been excluded from military maneuvers for approximately a decade.

The desert tortoise, *Gopherus agassizii*, is a federally-listed Threatened [USDI Fish and Wildlife Service (FWS) 1990] and California state-listed Threatened species [California Fish and Game Commission (CDFG) 1989] that inhabits the expansion area. The direction of base expansion became questionable following a 20 September 1991 draft Biological Opinion (FWS 1991) issued

by the FWS, which stated that base expansion to the south and southwest would jeopardize the continued existence of the species. To reinforce their opinion of this area as important to the recovery of the desert tortoise, the FWS designated much of the southern portion of the NTC, south to Interstate 40 as critical habitat for the desert tortoise (FWS 1994). Critical Habitat is defined in Section 3(5)(A) of the Endangered Species Act (Act) as "(i) the specific areas within the geographic area occupied by the species ... on which are found those physical or biological features (I) essential to conservation of the species and (II) which may require special management considerations or protection; and (ii) specific areas outside the geographical area occupied by the species at the time it is listed . . . upon a determination . . . that such areas are essential for the conservation of the species" (FWS 1994). The designation of Critical Habitat for a listed species is one of several protection measures aimed at aiding in the species recovery and its eventual removal from federal listing. It does so by focusing public and legal attention on areas considered important to a species' conservation.

In order to examine impacts to tortoise populations that might occur from NTC expansion, the Army directed several studies to identify tortoise abundance and distribution on and outside the southern NTC, beginning in 1988 (see Karl 2001 for review). Essentially, two types of study were employed for these purposes: plot studies and transects. Plot studies use labor-intensive, mark-recapture techniques to determine tortoise density (i.e., number of tortoises per unit area) at a single, small site (e.g., 1 km², 1 mi²). Transects use belt transects, typically 10 m (33-ft) wide by 2.4 km (1.5 mi) long, placed over broad areas at a sampling rate of one to sometimes two transects per 2.59 km² (1 mi²). These provide a general idea of regional tortoise abundance and distribution and identify areas for further study.

The transect studies completed on and around the NTC failed to provide the level of accuracy or precision necessary to identify impacts to desert tortoises from the expansion, largely due to their low sampling rate (see Karl 2001 and 2002 for a review of their limitations). Furthermore, no plot studies had been conducted in the western expansion area (Superior Valley Area). In response to the need to more precisely estimate impacts to the desert tortoise that might occur in the expansion area, seven plots were surveyed in 2001 in the western

expansion area (Karl 2002). In addition, an intensive survey using belt transects was implemented, where four transects per kilometer were completed on 145, generally adjacent, square kilometers within the expansion area. This increased the sampling intensity from the historic rate of 1.5 to 14.7 % of a square kilometer (0.6 to 5.9% of a square mile). Additionally, several improvements in data collection and analysis were employed to increase the accuracy and precision for estimating tortoise densities that were lacking in the earlier studies. Efforts were concentrated in the western expansion area because little was known about tortoises there. By contrast, the 9-0 Area was already part of the NTC and had experienced military training, so limited surveys were completed there. Six study plots and 440 transects were completed in the western expansion area; one study plot and 104 transects were completed in the 9-0 Area.

The 2001 plots used a Peterson estimator, where two, 100 percent surveys of each plot were completed, the first being the "marking" coverage and the second, the "recapture" coverage. Estimated tortoise densities on all of the plots were low to very low, 0-14 tortoises/ km² (0-36 tortoises/mi ²). Low densities, and the attendant low capture rates, resulted in an unfortunate statistical difficulty associated with low capture rates - broad confidence intervals (i.e., low precision) around the density estimates. intervals are the range of values, including the point estimate for the population, within which the actual density can be expected to occur. For instance, if the point estimate is 5.0 tortoises/km² and the confidence interval is 0.43 to 116.93 (Plot 2 from 2001), it can only be said statistically that the actual population estimate falls somewhere between 0.43 to 116.93 tortoises/km². So, while there was no doubt among biologists associated with the project that the densities were low on the plots (supported by low capture rates and low levels of tortoise sign), the point estimates from the markrecapture analyses could not be supported statistically. Since the point density estimates on the plots were the basis for determining tortoise densities on the subsequent transects and the resultant estimate of total tortoises in the western expansion area, it was necessary to demonstrate that the point estimates for the 2001 plot studies were reasonably accurate, despite the low sample size. In 2002, two plot studies were completed that employed multiple plot coverages (at least three), thus permitting the use of another statistical model to

determine tortoise density, in addition to a Peterson estimator. Multiple-coverage models can result in higher precision (smaller confidence intervals) and it was anticipated that the 2002 studies would provide the needed support for the expansion area tortoise abundance results obtained in 2001.

Transects were also continued in portions of the 9-0 Area and the Superior Valley Area where they were absent or the data unusable in 2001 or where 2001 results suggested that further transects were warranted.

This volume should be read with the volume on the 2001 studies (Karl 2002). This volume is a continuation of those studies and the latter is incorporated here by reference. The 2001 report also contains many critical analyses of the methods used herein and discussions of key issues, not repeated here.

3.0 METHODS

3.1 PLOT STUDIES

3.1.1 Site Locations and Choice Criteria

Two plot sites were chosen for study, one of which was Plot 8 from the 2001 studies (Karl 2002) (Figure 3). The second plot, Plot 9, was approximately 2.7 km southwest of Plot 8, immediately south of the NTC border. The Universal Transverse Mercator (UTM) map coordinates (North American Datum 83, Zone 11) of the northwestern corners of the plots are:

Plot 8 - Easting 0526000, Northing 3889600 Plot 9 - Easting 0522200, Northing 3887500

These two plots were chosen based on the results of earlier surveys (Karl 2002, Chambers Group Inc., 1996), which indicated that densities at these sites were the highest that had been found in the area. It was hoped that capture rates would be sufficiently high for the 2002 results to achieve their goals of validating the 2001 results.

3.1.2 Site Size

One square kilometer was the basic plot size for Plots 8 and 9, consistent with plots worked in 2001. The rationale for a plot size of 1.0 km² was explained in Karl (2001 and 2002) and was based on a comparison of different plot sizes previously used and their attendant difficulties with edge effects, effective coverage, and violation of model assumptions. Both Plots 8 and 9 were increased to 1.44 km² to increase the number of tortoises captured and resultant statistical precision.

3.1.3 Survey Techniques

Plot studies were completed between 8 and 27 May 2002. A 100 x 100 m, north-south, east-west grid was established on each plot prior to surveying for tortoises, in order to ensure the accuracy of both surveying the plots and mapping tortoise sign. This was accomplished using a Trimble Pro XRS, real-time, Global Positioning System (GPS) unit with sub-meter accuracy. Stakes numbered with UTM coordinates were planted every 100 meters. Each grid cell was assigned a consecutive number for identifying locations of tortoises, other tortoise sign, habitat features, and walking coverage (Appendix 1).

The mark-recapture surveys conducted on the plots entailed surveying the entire plot once, marking and releasing all tortoises found, then re-walking the plot two (Plot 8) to three (Plot 9) more times, tallying all new tortoises plus all tortoises previously marked (recaptures). Plot coverage was identical during both the capture and recapture samples, with the exception that grid cells were walked in opposite directions on successive passes (e.g., north-south, then east-west, etc.). Plots were worked from the center outward, to assess the completeness of the first survey and the point at which recapture rates declined. Every cell within the grid was completely walked, generally by a single person, using approximately 10-meter-wide transects. In most cases, cells were searched by different people on the mark and recapture samples. In all cases, each cell that was walked by a less experienced observer was subsequently walked by an experienced observer. Each plot coverage required approximately two days to complete, using a crew of nine people. The second (recapture) survey immediately followed the first to meet the closure assumption for the mark-recapture analyses (see Section 5.1.1 below). Completing capture and recapture surveys with a large crew also minimized lack of detection of tortoises as they moved through the plot. With only one to two searchers (the standard on many historical plot studies), a tortoise can remain undetected as it moves into areas already searched; but with a large crew, a larger portion of the plot is surveyed simultaneously, thereby minimizing the number of tortoises missed.

It is well-known among tortoise researchers that capture rates decline when tortoises are sequestered in burrows, which are often surprisingly well-

camouflaged. This can occur daily or seasonally in response to ambient temperatures, or seasonally to annually in response to drought. In Spring 2002, there was little forage because rainfall during the previous winter had been negligible. Tortoise activity was substantially diminished as a result. Still, we attempted to maximize the tortoise encounter rate by working primarily during those temperatures when tortoises would be active. We completed most of our searches when ground surface temperatures were less than approximately 43 C, the approximate temperature at which tortoises go underground to avoid lethal thermal conditions (Karl, 1992; Zimmerman *et al.* 1994). This resulted in a bimodal daily pattern of searching to coincide with the bimodal activity pattern of tortoises in May. Typically, the morning session began at approximately dawn or shortly thereafter and ended in midto late morning, resuming in mid- to late afternoon and ending at dusk. Weather data (air and ground surface temperature, wind speed, and cloud cover) were recorded hourly to monitor ambient conditions.

Tortoises were sought both above-ground and in their burrows, the latter using reflecting mirrors, flashlights, and blunt probes. Each tortoise was measured, weighed, described relative to shell wear and identifying characteristics, sexed, examined non-invasively for health conditions, photographed (Appendix 2), released at the capture site and its location described and mapped on individual grid cell forms (Appendix 3); all behaviors were noted. Every tortoise was also marked with an individual number. Temporary numbers, placed on both the anterior and posterior carapace so that the tortoise could be easily identified in its burrow during subsequent plot coverage, without disturbing it, were made of white correction fluid (e.g., "Wite-OutTM"). A more permanent identification mark was also placed on the carapace, in the likely event that there would be future studies in the area. A small dot (less than approximately 8 mm in diameter) of either white correction fluid or yellow acrylic paint was painted in the center of a rear scute where it would be subject to the least abrasion. A number was written in permanent ink on this dot and sealed with clear epoxy. All handling of tortoises was directed to minimize handling stress and prevent disease transmission to other tortoises, and was consistent with the Desert Tortoise Council's handling guidelines (Desert Tortoise Council 1994). personnel authorized to handle tortoises on Federal Recovery Permit No.

TE746058-6 and California Scientific Collecting Permit No. 801041-05 did so.

Numerous other data were also recorded on the individual grid cell forms (Appendix 3). All tortoise burrows and scat were measured, described relative to recency of use, and mapped. For tortoise burrows and scat, descriptions of age followed a key (Appendix 4) based on the principal investigator's previous observations (Karl, field notes). All tortoise remains were individually numbered and marked, measured, sexed (if possible), examined for cause of death and indicators suggesting time elapsed since death, mapped, and photographed (Appendix 5). Because coyotes (Canis latrans) and kit foxes (Vulpes macrotis) (Family: Canidae) are known predators of desert tortoises, all canid dens and burrows were mapped and described. Canid scat were also examined for tortoise remains. Common ravens (Corvus corax), also known to be predators of juvenile tortoises, were noted when observed. Finally, all anthropogenic surface disturbances were mapped and described.

During the recapture surveys of each plot, all tortoises, carcasses, burrows, and canid sign were sought in an identical manner to the first coverage of each plot, with the exception that tortoises and other sign marked during the first survey were not re-processed. These sign were, however, noted by location, number, and, if a tortoise, by behavior.

3.1.4 Data Analysis

3.1.4.1 Tortoise Density. Tortoise density on each plot was estimated using two mark-recapture models: Peterson and Schnabel. These two, mark-recapture models were chosen because (a) the Peterson estimator was used on the 2001 plots and (b) assumptions for both are met. The primary assumption is that the population is closed. In other words, there were no gains (from birth or immigration) and no losses (from death or emigration) during the study. Because each plot was surveyed completely (i.e., mark and recapture runs) in 7 to 11 days, and tortoise activity was depressed this spring because of lack of forage, this assumption should be true. Other mark-recapture models were rejected for assumption violations [e.g., Jolly-Seber (Jolly 1965)] or

insufficiently large sample sizes [Burnham-Overton (Burnham and Overton 1978)].

All mark-recapture models are based on the concept that the unknown value, population density, is a proportion of marked to unmarked animals. The Peterson estimator expresses this simply, as a proportion of the number of tortoises originally marked and recaptured to the total number captured on the second sample by the equation:

$$N/M = n/m \tag{1}$$

where:

N =the population estimate

M = the number of animals marked in the first sample
n = the total number of captures in the second sample
m = the number of marked animals in the second
sample

The modified, less-biased form of this estimator (Chapman 1951, Greenwood 1996) was used in 2001 and again used in 2002 for consistency:

$$N = [(M+1)(n+1)/(m+1)] - 1$$
 (2)

A third survey (second recapture run) was conducted on Plot 8 and a fourth on Plot 9, in order to apply a Schnabel estimator (Schnabel 1938). This method of calculation is based on the theory that as more animals are marked in successive surveys, the proportion of marked to unmarked animals increases, finally reaching 1.0. The number of marked animals at this point, then, is the estimated population size. The Schnabel formula for calculating an estimated population size is:

$$N = \frac{M_i n_i}{m_i}$$
(3)

where:

i = the sampling occasion

 M_i = the number of animals marked prior to

the ith sample

Because total recapture rates are <50 on both plots, 95 per cent confidence limits were derived from tables by Chapman (1948, in Overton 1971).

Both the Peterson and Schnabel methods are subject to assumptions other than the closure assumption discussed above. One is that marks must not be lost between samples. Our method of marking tortoises, as well as drawing each captured tortoise in detail, ensured this. Another assumption is of equal catchability, or observability, in the case of tortoises. Otis et al. (1978) describes three aspects of equal catchability: (1) whether captured tortoises behave the same as uncaptured tortoises such that all tortoises have the same likelihood of capture on recapture runs; (2) if there is a temporal effect on tortoise behavior, and resultant observability, between capture and recapture surveys (e.g., due to temperature, forage conditions); and (3) whether individual variation in tortoise behavior that would affect observability. This first aspect of equal catchability is true as tortoises do not exhibit a behavioral response to marking or handling, such as moving away from the capture area or becoming more secretive, that would make them more difficult to find in future plot coverages (pers. obs.; also see Turner et al. 1982, Freilich et al. no date). The second aspect was eliminated because all capture and recapture runs were completed in 6 to 11 days. The effects of individual behavioral differences on equal catchability are unknown, although there are likely to be some. Certainly, this third aspect of observability applies to tortoise size. Tortoises smaller than 140 mm in length are statistically less likely to be caught (Turner and Berry 1984, Karl 1989) due to their extremely low visibility. As such, density estimates were stratified into those for tortoises larger than 180 mm in carapace length, which is the reproductive cohort (Karl 1998a, Wallis et al. 1999), and those from 140 to 179 mm, 100 to 139 mm, and <100 mm in length.

3.1.4.2 Other Analyses. Annualized mortality (AM) rates were calculated for tortoises larger than 140 mm MCL, using the geometric mean of mortality

for both the two-year and four-year periods prior to, and including, Spring 2001:

$$AM_{2 \text{ yr}} = 1 - \begin{bmatrix} \frac{\text{# tortoises alive in } 2001}{\text{# tortoises alive in } 2001 + \text{# shells}} & 2 \text{ years old} \end{bmatrix}$$
 (4)

(The AM for the four-year period used shells 4 years old and the quarter-root.)

Only those shells that were considered to be individual animals were counted in the calculations. The use of carcasses to determine mortality rates is based on the assumptions that (1) as many carcasses are carried onto the study site by scavengers as are carried off the site; (2) partial carcasses can be reliably distinguished as individual animals (i.e., not a part of other partial carcasses); (3) all carcasses present can be detected; and (4) the age since death can be reliably determined. Assumptions 1 through 3 can be considered valid for larger tortoises, which was primarily the size group found on both plots. It is often difficult, however, to determine the period of time since the death, especially for tortoises that have been dead more than two years. Level of exposure to sun and the size of the tortoise are two factors that affect shell, bone, and scute quality and, hence, determination of the time of death. However, the more recent the death, the easier this determination becomes. Freshly dead tortoises or those that have died within the previous few months are easy to identify, and even the time since death for those that have died within the previous year to, sometimes, two years is fairly identifiable (Karl, field notes). An earlier, informal study, where the exact date of death was known in numerous tortoises of reproductive age that were subsequently monitored over six years following death (Karl, field notes), plus the carcass aging scheme presented by Woodman and Berry (1984), were used to estimate ages of carcasses.

3.1.5 Survey Personnel

Nine people participated in the surveys (plus a tenth the last four days), of which three had extensive experience searching for tortoises and their sign (Frank, Karl, and Vaughn), one was moderately experienced (Mueller), and the remainder were inexperienced relative to tortoises, but had slight to extensive field experience (Appendix 6). The least experienced participants were instructed in tortoise habits, sign, and search techniques prior to starting surveys, and were accompanied by experienced observers until it was determined that they were capable of locating tortoises. To ensure adequate plot coverage, inexperienced observers only walked grid cells that were adjacent to those covered by more experienced observers and never walked the same grid cells during the capture and recapture samples.

3.2 TRANSECTS

3.2.1 Transect Locations

The UTM grid map was used to divide the survey area into 1.0 km² cells. A total of 29 square kilometers was sampled by belt transects (Figure 3). Sampling was conducted in the valley north of the Paradise Range, where the most intensive military training is anticipated, and in the 9-0 Area. Site choice was based on the results of Year 2001 surveys (Karl 2002), and included areas where data gaps appeared, as well as some kilometers that were sampled in 2001, but for which the data were unusable. In addition, surveying in the 9-0 Area was extended to the north, to better estimate the impacts that will occur to the tortoise population from renewed training activities there.

3.2.2 Survey Techniques

Transects were 2.4 km (1.5 mi)-long and configured as equilateral triangles, consistent with earlier transects on and around the southern NTC. Four transects were walked in each kilometer, each systematically starting in a corner of the kilometer (Figure 4). All four transects in each kilometer were walked by a single observer. Direction was maintained with a hand-held, orienteering compass or GPS unit.

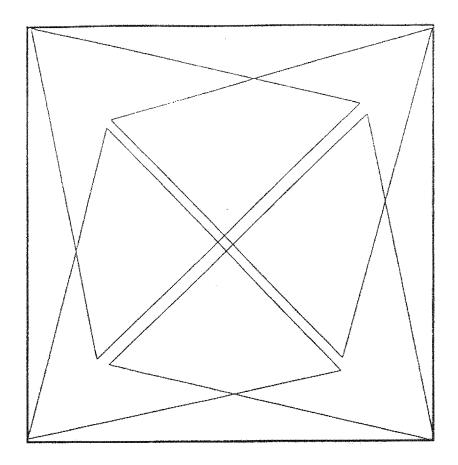


Figure 4. Transect configuration in each square kilometer sampled on the proposed expansion area. Each transect is 2.4 km long.

While walking each transect centerline, observers scanned back and forth across the transect centerline, attempting to observe all nearby tortoise sign. All tortoise sign was measured, mapped and described relative to gender, condition, and age, using the key found in Appendix 3. The perpendicular distance of each sign from the centerline was measured (by pacing) to 0.5 m. Current and recent weather conditions were recorded and the topography, drainage patterns, soils, substrates, plant cover, anthropogenic disturbances, and aspect-dominant, common and occasional plant species were described and mapped. Mapping sign and habitat features was achieved by tallying paces or using a GPS unit. All transect data were recorded on specially-designed forms (Appendix 7). Representative areas in each sampled kilometer were photographed. Both observers had spent the previous spring searching

for tortoises and their sign, so refreshing their search images was deemed an unnecessary exercise prior to conducting transects (as was done in 2001).

Transects were begun on July 10 and ended by July 27 This time period was chosen because the optimum time to conduct transects is summer, following the spring tortoise activity season, because of maximum sign accumulation then. This is especially valuable where tortoise densities are low.

3.2.3 Data Analysis

3.2.3.1 Calibration Transects. Transects are an indirect measure of tortoise density based on an index of tortoise sign counts to actual tortoise density. In order for each observer to develop his individual index whereby density could be estimated from tortoise sign on transects, four "calibration" transects were walked on Plots 1, 2, 4, 8, and 9. Each transect was systematically placed in the plot in the identical configuration as those on the remainder of the kilometers sampled in the expansion area (Figure 4). The mean number of sign (Y) for the four transects walked in each square kilometer was regressed on the tortoise density for that plot (X), using a simple linear regression analysis and forcing the regression through the origin (Figure 5):

$$Y = \beta N \tag{5}$$

where:

N =the population estimate

 β = the proportion or multiple of abundance

[Logically, if tortoise density is zero, then there should be no sign; i.e., the intercept at zero tortoise density is zero. There may also be a threshold density above zero tortoise density below which no sign is detectable; i.e., the intercept is negative. Because the intercept for different observers has been shown to be inconsistent (see Karl 2002), in some cases suggesting that sign would be present even if density were zero - i.e., the intercept is greater than zero - consistency and logic dictated forcing the regression through zero.]

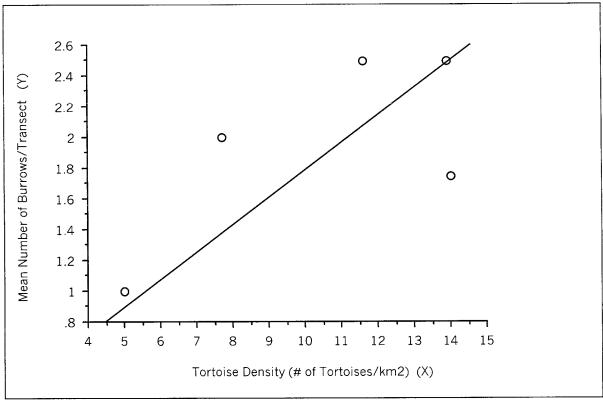


Figure 5. Example of regression of mean number of burrows per calibration plot (four transects per plot) on tortoise density (tortoises 180 mm in carapace length only) on five calibration plots (Observer: PF). Resulting equation is: Y = 0 + 0.179X; p = 0.001; $r^2 = 0.94$.

Solving Equation 5 for N (after Conroy 1996) yields:

$$N = Y/\beta \tag{6}$$

identifying 1/B as that constant, called the "calibration coefficient", by which each observer needed to multiply his sign counts on subsequent NTC expansion transects in order to provide an estimate of tortoise density (Table 1). [Note: Calculating calibration coefficients using mean sign counts per kilometer, rather than regressing the results of each transect on its relevant

TABLE 1

SUMMARY OF CALIBRATION TRANSECT RESULTS AND CALIBRATION COEFFICIENTS, BY OBSERVER

OBSERVER					BURROWS ^{1,2}	WS1,2					CALIBR COEFFI (CONFI INTER	CALIBRATION COEFFICIENT (CONFIDENCE INTERVALS) ³
	PLOT 1	<u>Г</u>	PLOT 2	Γ2	PLOT 4	T 4	PLOT 8	∞ ∞	PLOT 9	6]	MOT	HIGH
	Per	Mean	Per Mean	Mean	Per Mean	Mean	Per Mean	Mean	Per Mean	Mean		
	Transect		Transect		Iransect		Iransect		Tansect			100
	2-3	2.25-	7	 9	1-2	2.00-	3-5	3.50-	2-9	6.00	3.46	2.87
		3.25		1.50		2.50		4.50		6.25	(2.26-7.35)	(2.11-4.48)
	5-6		1		3		3-4		7			
	1-2		0-1		_		2		8			
	1-2		1-2		3-4		2-9		33			
	3	1.75	2	1.00	3	2.00	1	250-	3	2.50	5.59	5.46
	•							2.75			(4.18-8.47)	(4.00-8.55)
	2		1		2		4		5			
	2		0		0		2-3		2			
	C		1		3		3		0			

Only burrows representative of tortoises 180 mm in carapace length and within the truncated distance for

Appendix 3 for explanation of burrow classes.) "Low" correlation coefficient corresponds to low range of mean values. "High" calibration coefficient each observer are presented. Range in burrow sign and mean reflects inclusion of questionable tortoise burrows (high end). (See 7

corresponds to high range of mean values.

33.

half-kilometer density, was analyzed in 2001 studies (Karl 2002). This approach was found to be statistically more robust.] The r^2 values for the individual calibration coefficients were high, ranging from 0.87 to 0.94. R^2 , the coefficient of determination for the regression model, is a measure of the strength of the correlation between the independent and dependent variables, and the closer r^2 is to 1.0, the stronger the predictive value of the model.

A monotonic increase in the model slope is assumed, although there were no calibration plots with densities exceeding 14.0 tortoises per square kilometer. This assumption is biologically sound and supported by other transect studies with higher density calibration plots (e.g., Chambers Group, Inc. 1996). It should also be noted here that an argument can be made that the initial regression of sign on known tortoise density should be reversed, i.e., tortoise density regressed on sign. However, there is precedence for the former method as applied to tortoises (Berry and Nicholson 1984, Karl 1983, Chambers Group, Inc., 1994) and other species (Conroy 1996) and it is logical that sign is the response (dependent) variable. The calibration coefficients are similar for both approaches, albeit not identical. It should be kept in mind, however, that these are *indirect* estimates of abundance and, as such, are approximate by their nature. Small differences should not be considered biologically significant and most likely would not be statistically significant.

Despite the extensive experience of the observers, it could not always be definitively determined if each observed burrow belonged to a tortoise (See Appendix 4, Burrow Classes). As such, in some cases there was a range of burrow counts for a transect that included the total number of definite burrows at the lower end of the range and this number plus questionable burrows at the upper end. This resulted in a range for the calibration coefficients, as well (Table 1). When calibration coefficients were subsequently applied to expansion area transects where only definite tortoise burrows were observed, the calibration coefficient that resulted in the highest tortoise density estimate was used, in the interest of conservation. Where expansion area transects resulted in a range of total burrows, that included questionable burrows in the upper end of that range, the relevant calibration coefficient was applied to each burrow total in the range.

Calibration transects were conducted during the approximate middle of transect set for each observer, in order to maximize their applicability to transects done both early and late in the set. For other studies conducted in the area of the NTC expansion prior to 2001 (Karl 2002), calibration transects were conducted only at the beginning of the transect set.

3.2.3.2 Sign Type Used. While all tortoise sign observed on a transect was recorded, only burrows were used to estimate tortoise abundance. This is because the detectability of other sign is dependent on ambient temperatures (tortoises), season (tortoises), or recency or substrate (tracks, drinking depressions, and courtship rings); visible eggshell fragments are largely reliant on predator activity (Karl 1998a). Carcasses are only indicators of tortoise death, not the current abundance of live tortoises. With regard to scat, difficulties generally are related to the low and inconsistent visibility of scat. Burrows are statistically and logically more easily detected than are scat (Karl 2002), largely because of their greater size and the oft-visible mound. Scat are not only small, but their visibility is affected by substrate. On gravelly or dark surfaces, it is far more difficult to see scat than on pale and/or fine-gravelly substrates, a factor that does not affect burrow visibility. The lower visibility of scat is also apparent in the lateral distance from the transect centerline at which scat and burrows are observed. Karl (1989) observed that 65 % of 553 scat were seen within the first 0.5 m of the centerline; 75 % were seen within the first meter (Figure 6). By contrast, burrows were easily seen to approximately 5 m (Figure 7). While scat have been used as a predictor of tortoise density on all transects in the NTC area prior to 2001, adding scat counts to burrow counts on calibration transects was found to consistently lower the strength of the prediction for tortoise density in 2001 studies (Karl 2002). As a result, in both 2001 and 2002 transects, only burrow counts were used to estimate tortoise density.

3.2.3.3 Transect Width. Prior to 2001 studies, 10 m was standardly used as the pre-set transect width for all observers. This is an erroneous approach as it may either eliminate useful data (outside the 10 m width) or include unreliable data if the functional transect width is other than 10 m. Unreliable data here are sign that cannot be consistently seen and, therefore, lower the

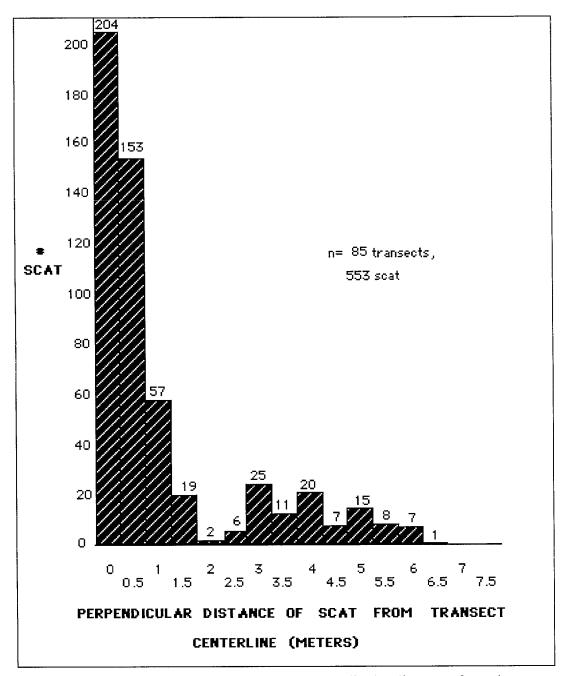


Figure 6. Frequency of scat sign at various perpendicular distances from the transect centerline.

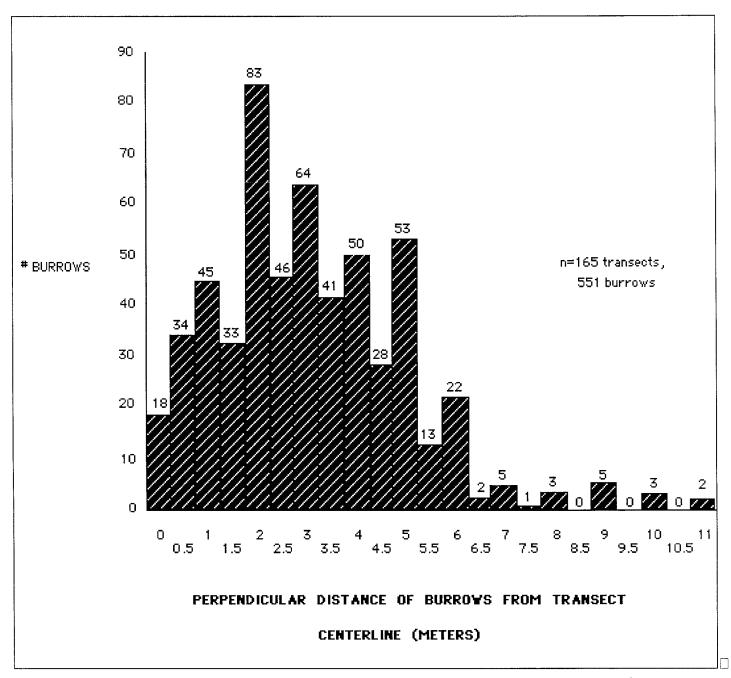
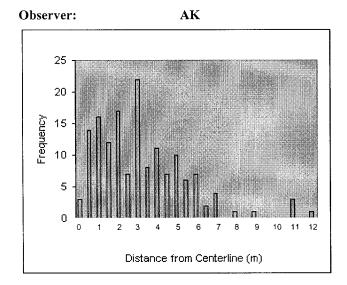
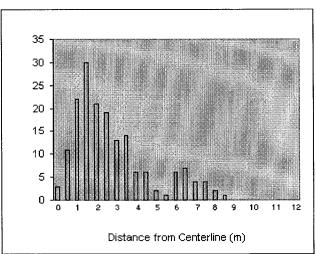


Figure 7. Frequency of burrow sign at various perpendicular distances from the transect centerline.

robustness of the predictor model. Inconsistently seen sign will vary by sign type (the major reason that scat were eliminated from the predictor model), but also by observer. In both 2001 and 2002, I let the data indicate where the appropriate data truncation should occur, instead of a pre-set 10 m. Since observers have different scanning patterns and search widths (Karl 2002), the best transect width for burrows was determined for each person using frequency histograms of every burrow observed (all transects) to its perpendicular distance from centerline (Figure 8). Such graphical representation of the lateral distance at which burrow visibility becomes inconsistent indicates the transect width for that person. To augment the estimate of transect width based upon the visual point of data truncation, outlier points were eliminated based upon elimination of 10 % of the sample points (after Buckland *et al.* 1993).





PF

Figure 8. Frequency histograms of scanning differences (i.e., distance that burrows are observed from transect centerline) between observers, for the purposes of truncating transect width to that belt width in which sign is reliably observed.

3.2.3.4 Sign Size. On plots, captures for tortoises smaller than 140 mm in carapace length have been shown to be statistically significantly lower than for larger tortoises (see Section 3.1.3.1, above). The probability of detecting small tortoises or their sign is even lower on transects. Only two of the

burrows seen on all transects were those of tortoises smaller than 180 mm in length. [Wilson et al. (1991) observed that burrow width approximately corresponded to tortoise length for gopher tortoises (Gopherus polyphemus). While burrow width tends to be somewhat greater than the length of the desert tortoise occupying the burrow (pers. obs.), this proportional value is unknown, so I used a 180 mm burrow width as the cut-off for adult tortoises. These results are similar to earlier results on NTC transects where 85 % of 874 burrows represented tortoises 200 mm in carapace length (Karl 2001). Furthermore, few tortoises smaller than 180 mm in length were found on the calibration plots, so no calibration coefficients could be determined for this cohort. The expansion area transects, then, apply only to the reproductive cohort of tortoises.

3.2.4 Survey Personnel

Two people conducted the transects (Appendix 6), each of whom was a highly experienced tortoise biologist with many years experience searching for tortoises and their sign.

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 VALIDATION OF EARLIER DENSITY ESTIMATES

Several conclusions are evident from this two-year study. Most critical is that the density estimates on the mark-recapture plots completed in 2001 are valid. This conclusion is based on both the repeatability evident in the results from 2001 to 2002 and the repeatability among results obtained using different models. So, the point estimates on which the transect indices, and ultimately the abundance in the expansion area were based, can be assumed to be approximately correct. While multiple recapture periods narrow the confidence interval for the estimate, the estimate doesn't change. The western area abundance estimate reported for 2001 of less than approximately 1000 adult tortoises (Karl 2002) and the western and central 9-0 Area estimate of approximately 400 adults is likely to be fairly accurate.

5.2 IMPROVEMENTS IN TECHNIQUES

Historically, tortoise transects used to estimate regional tortoise densities have been poor predictors of tortoise abundance (see review in Karl 2001 and 2002). The current study incorporated numerous improvements in both the transect and plot methodologies that were discussed in detail in Karl (2002). They included factors such as sampling intensity (increased from 1.47 % to 14.7 % of a square kilometer), tortoise size cohort considerations, use of appropriate sign types for the density index, data truncation by sign type and observer, identical sampling intensity per kilometer for both calibration transects and expansion area transects, and appropriate choice of plots for calibration transects. All of these factors resulted in high regression correlations for the index (i.e., r^2 values), thus providing high confidence in the ability of these transects to predict tortoise density. This, combined with the year-to-year consistency in the transect results strongly validates the reliability of the revised techniques.

5.3 CONSERVATION ISSUES

The 9-0 Area has a substantially higher population than is present in the western expansion area. Notwithstanding that the FWS declared both the area abutting the 9-0 Area and the Superior Valley Area as critical habitat (the FWS 1994), there is no evidence that the latter contains the constituent elements essential to conservation of the species. Not only are tortoise densities low to very low throughout the area, but it is likely that densities were probably never high there, based on habitat and elevational influences (see Karl 2002 for discussion).

By contrast, the 9-0 Area has moderate densities that are more consistent over the area and better habitat than the western expansion area, so it may have supported higher densities than it currently does. Current densities are likely to be depressed due to high mortality and depressed reproduction associated with the recent, 14-year drought (see discussion in Section 4.1, above). In the 9-0 Area, Chambers Group, Inc. (1994) estimated that there were 5000-6000 tortoises in 1993. This abundance was calculated from the results of transects (sampling rate: 0.77 transects/km²) that estimated broad areas to have in excess of 39 tortoises/km² and even 97 tortoises/km². This is substantially higher than the 2001 and 2002 estimates, when the maximum density predicted for this area was generally <17 tortoises/km², although it reached 33.0 tortoises/km² in one corner. If these differences are accurate and not attributable to differences in techniques and analysis, they would suggest a high, continuous mortality rate or high rates with pulses of very high rates.

Tortoise densities south of the 9-0 Area may not be as high as those on or immediately below the 9-0 Area because of the poorer habitat quality throughout much of that area [pers. obs. and West Mojave Interagency Planning Team (WMP) 2000]. While the difficulties associated with the WMP's (2000) techniques of data collection and interpretation (see Karl 2001 and 2002) make the results somewhat questionable, the WMP identifies the Mud Hills area northwest of Barstow as a high tortoise density area. In combination with the 9-0 Area, then, it is these two population centers that are probably most important to the conservation of the species in the Superior-Cronese Critical Habitat Unit.

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